Red Blood Cells go ECO: The Extended Cut

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Enzymatic conversion of A and B erythrocytes to blood group O brought a step closer by exoglycosidases isolated from the mucolytic gut bacteria *Akkermansia muciniphila*.

The ABO blood group antigens consist of sugars, also known as glycans, located on the lipids and membrane proteins present on the surface of red blood cells (RBCs). Among these, type O is the most clinically important because individuals with this blood group have only the H antigen produced by a fucosyltransferase and lack the enzymes required to add the specific sugars that convert the H antigen to type A and/or type B blood groups. This characteristic makes blood from group O individuals the most universally compatible ABO blood type[1]. An increased availability of blood of this blood group type is crucial for emergency situations where time or resources do not allow for typing. Consequently, efforts to enhance the availability of blood group O RBC by removing the sugars from A or B antigens has been a longstanding pursuit within the blood transfusion clinical research community[1]. Much of this endeavour has been concentrated on the concept of enzymatically converting A and B type RBCs into group O, referred to as "Enzymatically Converted Group O" or "ECO-RBC".

Now, writing in *Nature Microbiology* Jensen et al[2] report the discovery of a new blend of exoglycosidases sourced from the dedicated mucin-degrader bacteria *Akkermansia muciniphila* that can transform both A and B antigens and crucially their extensions to generate ECO-RBC with high compatibility with group O plasma. Confirmation of the under-recognised antigenicity of extended A and B glycans alongside discovery of a suite of glyco-conjugate converting enzymes amongst the mucolytic gut microbiota that can cleave such extensions represents an important step toward utilisable ECO-RBC.

The quest for development of an enzymatic treatment able to convert group A or B red blood cells to the universal donor group O (ECO-blood) is not a new one. Demonstration of enzymatic removal of Group B antigens by the coffee bean α-galactosidase in the early 1980s led ultimately to clinical trials that showed persistence of ECO-RBCs in circulation without adverse events[3,4]. Despite this success, safety concerns in the form of unexplained crossmatch reactions with recipient plasmas and an increase in B antigen antibodies after transfusion halted further progress[4]. The path toward replicating this partial success for the more prevalent blood group A has proved even more challenging. Subtypes of blood group A (A1 and A2), characterised by differing antigenicity[5], and the discovery of A antigen extensions[6-8] provide additional potential confounders for efficient conversion. After a long hiatus, a novel bioprospecting approach, involving the screening of a metagenomic library derived from the gut flora of an AB donor, led to the discovery that a two enzyme combinatory approach using GalNac deacetylase and galactosaminidase from *Flavonifractor plauti* could be used for A antigen removal , as assessed by flow cytometry and agglutination assays with anti-A antibodies[9], sparking renewed promise for this approach.

In this new study, Jensen and colleagues[2] report the discovery of enzymes with specificities, and also 2 enzyme structures, that allow for conversion of both core A and B glycan antigens, as well as their less antigenically characterised extensions. Through conducting extensive crossmatching studies they demonstrate the antigenicity of these extended structures, presenting convincing evidence that incomplete antigen removal is a likely factor in the unexplained blood group O plasma incompatibility for ECO-RBCs. By employing a clever strategy that focuses on identification and characterisation of the natural complement of exoglycosidases from intestinal mucolytic microbiota they succeed in assembling a blend of enzymes with high efficacy in removal of core and extended A or B antigens. This one pot approach for conversion to O can be performed at high haematocrit, within 30 minutes at room temperature and is potentially scaleable, key criteria with a view to future clinical application.

While the work reported here undoubtedly represents a major step forward, both in our understanding of the components that underlie ABO system antigenicity and as a blueprint for identification and exploitation of novel enzymes, there are still obstacles to overcome in the generation of truly O plasma compatible ECO-RBC remain.

The commendably thorough crossmatching studies highlight that only a small number of crossmatch positive tests post B antigen removal illustrating the success with converting the B type antigen. However, despite the reduction in antigenicity demonstrated to occur by dual removal of core and extended A structures, around half of triple enzyme treated blood group A RBCs reacted (to differing degrees) with O plasma. These data raise the complex spectre of possible enzyme-mediated exposure of cryptic neo antigens and, without diminishing the achievements presented, highlight the many factors that must be considered as blends of enzymes for improved conversion efficacy are developed, as well as the importance of employing pre-clinical crossmatching with broad O plasma panels.

In the long running pursuit of enzymatically converted blood group O RBCs, the work of Jensen et al[2] represents an important advance with targeting of canonical and extended A or B antigens set to become a feature of any successful future strategy. This comprehensive approach, involving the screening of a vast array of enzymes from *Akkermansia munciniphila*, coupled with extensive enzyme characterisation and in-depth structural analysis, sets a new precedent for identifying novel enzymes that could further enhance conversion efficacy. Moreover, the new enzyme structures, with their structural nuances and features has the potential to provide valuable insights into the mechanisms by which these enzymes interact and modify ABO antigens on RBCs. This, in turn, could lead to the future development of more specialised and effective enzymes. This not only holds great promise for more effectively converting RBCs possessing A or B antigens and their extensions into ECO-RBC but also serves as a platform for designing the next generation of enzymes through innovative protein engineering, reducing the risk of inadvertent exposure of neoantigens caused by existing enzymes and thus potentially mitigating antigenicity issues in a subset of the crossmatching studies.

Harnessing the evolutionary specialism of gut microbiota exoglycosidases has already uncovered new insights into the complex basis of ABO antigenicity. We await with interest the new insights and avenues for ABO universal blood that these studies promise for transfusion medicine.

Accompanying figure
Please can I request that we use an adapted version of Figure 1A from the Jensen et al manuscript but with the explanation of the enzyme reactions removed and replaced with the key exoglycosidases identified here from *Akkermansia muciniphila*.

We may need the authors to suggest where on the diagram the various enzymes act as its hard to follow in the manuscript.

Legend to accompany the adapted figure: The ABO blood group antigens on red blood cells and the sites of the *Akkermansia muciniphila* enzyme action. The illustration adapted from Jensen et al Figure 1A, showing the predominant blood group A,B and H type 2 antigens on red blood cells as well as the less abundant H type 1 and 4 chains. The three extensions of A antigen (Gal-A, Htype 3 and A type 3) and the extended B antigen GalNAc-B are also depicted.

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